(10-90 mM) were inhibitory. Further, this activating mechanism was not intrinsic to the PC2 channel but instead seems to be mediated by PC2-associated proteins. Channel function of the *in vitro* translated PC2 protein with Ca^{2+} concentrations of 10-15 mM was non-responsive to lowering cytoplasmic Ca^{2+} with either EGTA or BAPTA. Our data are consistent with a regulatory role of both cytoplasmic and external Ca^{2+} in PC2 channel function, which does not involve putative Ca^{2+}-binding sites on the channel protein, but instead external sites to the channel protein.

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Molecular Basis of Calmodulin and Ca^{2+} interaction with thermoTRP channels

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The TRP (transient receptor potential) family of proteins are relatively non-selective cation channels, most of which are highly permeable to Ca^{2+}. They participate in many sensory and physiological processes, and subdivided into seven major subfamilies: TRPV, TRPA, TRPC, TRPM, TRPP, TRPML, and TRPN. They are predicted to have six transmembrane domains per subunit, intracellular N- and C-termini, and form tetrameric assemblies. Several TRP channels have been proposed to be regulated by intracellular Ca^{2+}, and/or Calmodulin (CaM). Immunoprecipitation of TRP channels expressed in cultured cells suggests that CaM forms complexes with some of them, but questions remain about whether full-length TRP channels bind CaM directly or through some intermediary protein(s), and about the affinity and kinetics of complex formation. We have used fluorescence emission anisotropy of Alexa 488-labeled CaM to measure binding of CaM and Ca^{2+}-CaM to full-length TRP channels expressed in yeast and purified to homogeneity in detergent solution. At nanomolar concentrations, CaM bound rapidly to TRPA1 and to purified ryanodine receptor used as a positive control. CaM binding was enhanced by Ca^{2+}, but not strictly dependent on it, and binding of labeled CaM was antagonized by addition of unlabeled CaM. Dissociation of the complex occurred much more slowly than association. Under conditions in which TRPA1 and ryanodine receptor bound Ca^{2+}-CaM with high affinity, no binding of Ca^{2+}-CaM was observed for TRPV1 or TRPV2.

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Rapid Temperature Jump by Low Cost Laser Diode Irradiation

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Thermal TRP ion channels play important functions in somatosensation and pain. Aside from activation by agonist or voltage, they are directly gated by temperature, a novel property unique to these channels. However, the underlying mechanism of the abnormally high thermal sensitivity has remained elusive. Studies on thermal activation of these channels have been challenging, partly because of the difficulty for rapid alteration of ambient temperature in live cells. Existing approaches mostly employ resistive or thermal electric heating/cooling, and have a response time far slower than channel activation. As a result, they only allow for measurement of steady-state properties of the channel. We report here an alternative approach for rapid perturbation of temperature. The approach employs an infra-red laser diode as a heat source, and by restricting laser irradiation around a single cell, it can produce constant temperature jumps over 50oC in sub-milliseconds. Experiments with several heat-gated channels (TRPV1-3) demonstrate its applicability as a general tool for local temperature stimulation of single cells. Compared to other laser heating approaches such as those based on Raman-shifting of the Nd:YAG fundamentals, it is more cost-effective while providing adequate resolution for detection of ion channel kinetics.

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Heat Activation of Temperature-Gated Ion channels Studied by Fast Temperature Jumps

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Temperature-gated ion channels are distinct for their extraordinarily high thermal sensitivity. Activation by temperature is made possible with large changes in enthalpy and entropy and appropriate compensation between them, yet the source of the energy and the molecular basis of temperature sensing have remained elusive. The present knowledge on the thermal gating of these channels has resulted largely from equilibrium measurements of temperature responses. Nonsteady-state properties of the gating have been lacking because of the difficulty in rapid perturbation of ambient temperature. To address these issues, we have developed a laser-based fast-heating approach and used it to investigate the heat activation of the vanilloid receptor TRPV1. In response to a temperature step, the channel was activated with an exponential time course, and the responses were sustained at hyperpolarization but became slowly inactivated at depolarization. The rate of activation was variable depending on cell conditions, but had a time constant generally on the order of milliseconds. The activation showed saturation in both kinetics and steady-state currents at temperatures above 50oC. Strong activation occurred in saturating temperatures independent of membrane potentials. Nonlinear relaxation behavior was observed especially during the deactivation process under extreme hyperpolarization. Our data indicate that, despite large energetic changes, the temperature activation can occur rapidly, and consists of multiple states involving both temperature-dependent and independent transitions. The results provide further constraint on the locality of the energetic distributions and novel insights for understanding the mechanism of temperature sensing.

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Controlling Temperature and Chemical Environment for Patch Clamp Studies of TRP Channels

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A microfluidic device fabricated in PDMS, which permits control of local temperature and rapid exchange of solution around a single cell, has recently been developed. The system allows an experimental design where a single cell can be exposed to a large set of temperatures and concentrations of chemicals in a short time.

Transient receptor potential (TRP) channels are activated by both ligands and temperature, and synergetic effects exist between temperature and concentration of ligand. Therefore, together with patch clamp recording, the microfluidic system is used for studying the response and cooperative effects of temperature and chemicals in TRP channels.

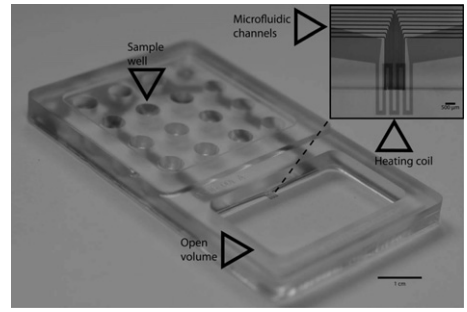

Figure 1. The microfluidic device contains 16 wells connected to separate channels, with separate outlets in the open volume. In the immediate vicinity of the outlet, there is no mixing between the flows from the different channels due to laminar flow. A thin-film Cr/Au-structure is aligned beneath the channel outlets, which yields resistive heating when a voltage is applied.

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Single-Cell RT-PCR Analysis of TRPC Channels Expressed in Rat Cholinergic, Dopaminergic, Noradrenergic, and Serotonergic Neurons in the Brain

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The canonical transient receptor potential (TRPC) channels comprise a family of nonselective cation channels composed of seven members (TRPC1-7). TRPC channels are widely distributed in the nervous system and contribute to neuronal excitation. In the present study, using the single-cell RT-PCR method, the distribution of TRPC channel mRNA is analyzed in cholinergic neurons in the nucleus basalis (NB), serotonergic neurons in the dorsal raphe nucleus (DRN), noradrenergic neurons in the locus coeruleus (LC), dopaminergic neurons in the substantia nigra (SN), and dopaminergic neurons in the ventral tegmental area (VTA). NB, LC, SN and VTA neurons are cultured from 3-5 day-old and DRN from 10-12 day-old Long Evans rats. Single-cell RT-PCR was performed on these cultured neurons using the previously described method (Kawano et al., 2004, Neuroscience letters, 358:63). Tyrosine hydroxylase primers were used to identify noradrenergic neurons in LC